(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 January 2004 (22.01.2004)

PCT

(10) International Publication Number
WO 2004/007554 A1

(51) International Patent Classification[7]: C07K 16/18

(21) International Application Number:
PCT/KR2003/000544

(22) International Filing Date: 20 March 2003 (20.03.2003)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2002-0041771 16 July 2002 (16.07.2002) KR

(71) Applicant *(for all designated States except US)*: **EYEGENE INC.** [KR/KR]; #187-114, Yonhee-3-dong, Sodaemoon-gu, 120-113 Seoul (KR).

(72) Inventors; and

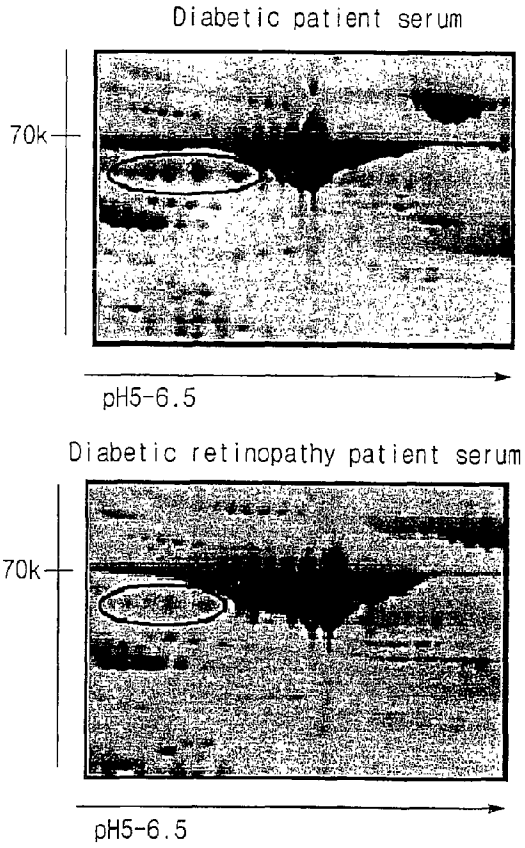
(75) Inventors/Applicants *(for US only)*: **YOO, Won Il** [KR/KR]; #212-1005 Chunggu Apt., Yangji-maeul, 36 Soonae-dong, Bundang-gu, Sungnam-si, 463-020 Gyeonggi-do (KR). **LEE, Sung Ho** [KR/KR]; #1403-1103 Okbit-maeul, Hwajung-dong, Duckyang-gu, Goyang-shi, 412-745 Gyeonggi-do (KR). **PARK, Kunewoo** [KR/KR]; 117-2201 CG Metrocity, Yongho-dong, Nam-gu, 608-090 Busan (KR). **CHO, Yang Je** [KR/KR]; #106-204 Samsung Raemian Apt., Dowon-dong, Yongsan-gu, 140-070 Seoul (KR). **AHN, Bo Young** [KR/KR]; 80-135 Bukgazwa 2-dong, Seodaemoon-gu, 120-132 Seoul (KR). **KWON, Oh Woong** [KR/KR]; #1906-1302 Woosung Apt., Gangsan-maeul, Ilsan-gu, Goyang-shi, 411-370 Gyeonggi-do (KR).

(74) Agent: **HWANG, Eui In**; 10th Floor, Hankook Tire Bldg., 647-15 Yoksam-dong, Gangnam-gu, 135-723 Seoul (KR).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK,

[Continued on next page]

(54) Title: PROTEIN FOR DIAGNOSING DIABETIC RETINOPATHY



(57) Abstract: The present invention relates to material for diagnosing Diabetic retinopathy. More particularly, the present invention relates to Immunoglobulin A protein for diagnosing Diabetic retinopathy, kit for diagnosing Diabetic retinopathy comprising antibody against the protein and method for diagnosing Diabetic retinopathy. The present invention can be used as diagnosing Diabetic retinopathy.

WO 2004/007554 A1

LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *with amended claims*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PROTEIN FOR DIAGNOSING DIABETIC RETINOPATHY

[Technical Field]

The present invention generally relates to diagnostic substances for diabetic retinopathy, and more specifically, to a diagnostic kit including an Immunoglobulin A protein and an antibody thereof, and a diagnostic method using the same.

[Background of the Invention]

In general, diabetes mellitus as a complex metabolic disorder causing microangiopathy is one of systemic diseases which broadly impair systemic tissues. Diabetes may affect vision, and most importantly, damage to blood vessels inside the eye (LEE Tae-hee, CHOI Young-gil. Diabetic Vascular Complications, Seoul: Koryo Medicine). Diabetic retinopathy, one of the most severe complications, becomes an important problem as life span and prevalence period of diabetic patients become longer due to improvement of living standards and development of treatment (Klein R. et al., Arch Ophthalmol. 102:520-532(1984)). Diabetic retinopathy has two stages a nonproliferative stage and a proliferative stage. The nonproliferative stage is characterized in that retinal lesions resulting from vascular disorders are limited in retina. The proliferative stage is characterized by penetration of neovascularization tissues from retina into the vitreous cavity (Green, In: Spencer WH, ed. Ophtalmic Pathology: an atlas and textbook. 4th ed. Philadelphia: WB Saunder; 1124-1129 (1996)). Diabetic retinopathy is diagnosed by observation of characteristic changes in the fundus structure. Vision loss due to diabetic retinopathy results from haemorrhagia corporis vitrei and maculopathy with traction retinal detachment of

yellow spot in the proliferative stage. Laser treatment with surgery treatment is well-known for its effectiveness for the vision loss (Diabetic Retinopathy Study Report Number 14: Int Ophthalmol Clin. 27:239-253(1987)). This treatment following proper steps can prevent vision loss, minimizing side effects. Diabetic retionpathy should be frequently examined and diagnosed to determine whether operation is performed on the diabetic retinopathy or not. However, since diabetic retinopathy is currently diagnosed only by funduscopy, it is difficult to detect diabetic retinopathy in its early stages. As a result, it is highly frequent for patients to miss opportunities to prevent the diabetic retinopathy and have an operation on it. Accordingly, a method is disclosed for diagnosing diabetic retinopathy easily in blood. There has been no method for diagnosing diabetic retinopathy using blood. The present inventors found a protein which varies in blood by using proteomics, and applied the protein to diagnosis. Since this protein shows a marked quantitative change between a diabetic patient with no diabetic retinopathy complication and a diabetic patient having a complication, the present invention comprising this protein is completed using accurate quantification by immunological method

[Detailed Description of the Invention]

In order to overcome the above-described problems, the present invention has an object to provide a useful diagnosis for diabetic retinopathy.

The present invention has another object to provide a kit for diagnosing diabetic retinopathy including the diagnosis.

The present invention has still another object to provide a method for diagnosing diabetic retinopathy.

In order to achieve the above-described objects, there is provided an immunoglobulin A protein, which is effective for diagnosing diabetic retinopathy, and a protein fragment thereof.

A sequence obtained by protein analysis corresponds to a constant site of immunoglobulin A heavy chain. The immunoglobulin A protein exists as heavy-chain and light-chain types. Since each chain has a variable region, the protein has sites having many different sequences. As a result, protein having a sequence, which may be determined as an immunoglobulin A protein, can be obtained.

The disclosed immunoglobulin A protein may have various amino acid sequences as well as SEQ ID NO:1 of heavy chain.

The amino acid sequence of H chain of Ig A is as described in SEQ ID NO:1.

The disclosed protein fragment of the immunoglobulin A can have various types of fragment including a peptide of SEQ ID NO:2.

There is provided an antibody specifically binding the protein. The antibody may be both polyclonal and monoclonal, but more preferably monoclonal.

There is also provided a kit for diagnosing diabetic retinopathy including the antibody.

The disclosed kit further comprises the antibody protein obtained by conjugating with enzyme peroxidase, alkaline phosphatase or biotin.

The rest reagents used in the disclosed diagnosis kit can be easily obtained from ingredients used in general diagnosis kits.

There is also provided a method for diagnosing diabetic retinopathy, comprising: a) treating the antibody with a blood sample and an anti-Immunoglobulin A protein

conjugated with peroxidase, alkaline phosphatase or biotin; and b) measuring optical density of the compound, wherein diabetic retinopathy is diagnosed when the measured result represents below 400mg/dL immunoglobulin A.

There are provided an Immunoglobulin A gene of SEQ ID NO:3 for coding an immunoglobulin A protein and a nucleotide of SEQ ID NO:4 for coding a peptide of SEQ ID NO:2.

Hereinafter, the present invention will be described in detail.

In the present invention, the immunoglobulin A protein of vitreous body in eyeball of diabetic retinopathy patients is shown to increase than that in healthy vitreous body. Here, the present inventor found that the protein changed in blood, that is, the immunoglobulin A protein of diabetic retinopathy patient decreases in blood than that of diabetic patients. Accordingly, a diagnosis for diabetic retinopathy is disclosed using an immunologic method.

In order to accomplish the above-described object, protein groups, which show specific changes to diabetic retinopathy, are analyzed using a proteomics method. The following results are found by analyzing quantitative changes of the proteins and the types of proteins in vitreous bodies of diabetic retinopathy patients and normal vitreous bodies. After the changes of the target protein is checked in blood, a kit for diagnosing diabetic retinopathy is prepared using a proper immunological method. First, a normal vitreous body is settled as a control group. The protein groups, which show qualitative and quantitative differences, are isolated in vitreous bodies obtained from diabetic patients and diabetic retinopathy patients by two-dimensional gel separation and image analysis. The protein groups are identified using MS and Q-TOF analyzers. The

protein wherein changes were observed and identified is proved as Immunoglobulin A. Increase of Immunoglobulin A, which is hardly observed in normal vitreous body, of vitreous body in diabetic retinopathy patients was observed. However, it has not been reported that immunoglobulin A increases in vitreous body of diabetic retinopathy patients. Second, this protein showed quantitative changes in blood. When blood of diabetic patients is a control group, immunoglobulin A decreases in blood of diabetic retinopathy patients. However, this result has not been reported, either. Third, a easy, sensitive and precise method for measuring existential values of proteins is selected by preparing a kit via an immunological method.

Hereinafter, the present invention will be described in detail according to preferred embodiments.

[Brief Description of the Drawings]

Fig. 1 is a diagram illustrating a process for pre-processing vitreous body of eyeball to be applied to proteomics.

Fig. 2 shows gel pictures illustrating CBB-stained fundus vitreous body proteins after two-dimensional electrophoresis. The proteins are not showed in the marked region in a vitreous body of normal eyeball while the proteins are showed in the marked region in a vitreous body of diabetic retinopathy eyeball.

Fig. 3 shows CBB-stained gel pictures illustrating serum proteins of a diabetic retinopathy patient and a diabetic patient alone, the proteins CBB-stained after two-dimensional electrophoresis. The excessive amount of protein exists in marked region for diabetic patient alone while the decreased amount of protein be showed in the

marked region for diabetic retinopathy patient.

Fig. 4 shows a graph illustrating the mass spectrum (A) of peptides treated with trypsin among proteins of the marked region of Fig. 2 using MALDI-TOF and Q-TOF analyzer, and the amino sequences of the peptide among the peptide fragments(B).

Fig. 5 shows a standard titration graph illustrating 0, 15.6, 31.25, 62.5, 125, 250, 500ng/ml immunoglobulin A standard solution and measured optical density values after ELISA reaction.

[Preferred Embodiments]

Example 1: Sample preparation of vitreous body for analyzing proteomics

Diabetic retinopathy is one of complications resulting from long-term diabetes. Diabetic retinopathy is characterized by generation of many abnormal neovascular systems having incomplete vascular structures, which causes bleeding in vitreous body of eyeball. The bleeding results in abnormality in retina, and further weakness and loss of eyesight. In the present invention, disease indicator was searched, and information on proteins for representing disease state was obtained by analyzing proteins in vitreous bodies of a normal control group and diabetic retinopathy patients, using a proteomics method. First to apply the proteomics method to the proteins, vitreous body was treated to be easy to analyze. The vitreous body contains large amount of high molecular weight mucopolysaccharide, hyaluronic acid. However, this polysaccharide was proved to interrupt protein separation. As a result, a method was devised to remove this polysaccharide effectively (see Fig. 1). First, 4ml vitreous body was diluted with 16ml distilled water, and put the diluent in a tube having a cut-

off membrane of 1,000,000 and centrifuged 8,000rpm at 4°C for 2 hours. This procedure was repeated three times to filter high molecular weight polysaccharide over 1,000,000 by difference of molecular weight. The non-filtered proteins were put in a tube having a 10,000 cut-off membrane and centrifuged 4,000rpm, at 4°C and then concentrated for analysis. The method for removing high molecular weight polysaccharide in the present invention enabled effective analysis by solving the problem that was not easily isolated in low pH.

Example 2: Investigate of protein groups changed in vitreous bodies of eyeball obtained from normal person and diabetic retinopathy patient

Proteins were isolated from each vitreous body and concentrated at 1mg/mL for analysis. First, the proteins were two-dimensionally separated by a stepwise method using two different characteristics of proteins. In the first step, proteins were moved according to net charge of the proteins by applying electrical stimulus to the proteins (IEF, pH 3-10). In the second step, proteins were moved on acrylamide gel (8~18%) according to molecular weight of each protein. One-dimensional electrophoresis (protein movement according to pH) was performed on the proteins with 50mA per gel for 12 hours. Then, two-dimensional electrophoresis (protein movement according to molecular weight) was performed on the proteins on poly-acrylamide with 50mA per gel for 6 hours. These moved proteins were stained with a Coomaasie Brilliant Blue-G250 stain and a silver-staining method for visualizing. The difference of proteins between in normal vitreous body and in vitreous body of diabetic retinopathy patient was analyzed by using image analysis software, Phoretix (Nonlinear dynamics, UK), in computer.

From analyzing the proteins in two groups, the present inventors confirmed that the protein group showing a difference existed (see Figs. 2 and 3).

Example 3: Identification of serum proteins that show the difference between diabetic patient and diabetic retinopathy patient

Proteins having differences in quantity and quality were searched and identified by MALDI-TOF and Q-TOF analyzers to know kinds of the proteins (see Fig. 4). It was shown that the amount of immunoglobulin A decreased in blood of diabetic retinopathy than blood of diabetic patient.

Example 4: Diagnosis of diabetic retinopathy by enzyme-linked immunosorbent assay(ELISA)

The present study was performed to find out whether serum of diabetic retinopathy among diabetic patients could be distinguished by Sandwich enzyme immunosorbent assay (ELISA) using anti-immunoglobulin A antibody. Serums were obtained from 10 normal healthy persons, 45 diabetic patients having no diabetic retinopathy and 86 diabetic retinopathy patients in hospital. First, 100ul of anti-immunoglobulin A (Koma, Korea) (1ug antibody protein per well; final concentration 10ug/ml) dissolved with coating buffer (50mM NaHCO3, pH 9.0) per well was reacted and coated in a EIA 96 well plate at room temperature for 1 hour. The each well was washed twice for 10 minutes with 400ul PBST, and then post-coated with PBS including 1% BSA. 100ul Serum of patients diluted with PBST buffer was put to the each well, reacted for 1 hour, and then washed five times with PBS. 100ul of diluted peroxidase conjugated-anti-immunoglobulin A antibody (KOMA Biotech Inc., Korea) was put into the each well, and then reacted for 1 hour. After

reaction, the each well was washed three times with PBS. Then, 100ul 0.1M citrate-phosphate (pH 4.9) containing 1mg/ml OPD (O-phenylenediamine dihydrochloride) and 0.03% H_2O_2 was put therein, and reacted at room temperature for 20~30 minutes. The reaction was stopped by 100ul of 3M sulfuric acid, and optical density was measured at 450nm using an ELISA reader. The amount of immunoglobulin A per blood unit volume (ml) was determined through applying conversion by standard titration curve and dilution rate to the optical density (see Fig. 5). As a result of ELISA measurement, the amount of immunoglobulin A ranged from 131.2 to 298.7 mg/dL in serum of normal person, from 226.5 to 771.9mg/dL in serum of diabetic patient, and from 105.3 to 557.2mg/dL in serum of diabetic retinopathy patient. These results were shown as average values in Table 1. As the measurement average value of immunoglobuline A, 217.6 ± 82.1mg/dL was shown in normal person, 457.6 ± 151.6mg/dL in diabetic patient, 244.4 ± 117.1mg/dL in non-proliferative diabetic retinopathy patient, and 278.6 ± 123.6 mg/dL in proliferative diabetic retinopathy. Here, it was remarkably shown that the large amount of immunoglobulin A existed in serum of the diabetic patient group. However, it was shown that there was little difference in the amount of immunoglobulin A in serum of non-proliferative and proliferative diabetic retinopathy patient. If diabetic retinopathy was decided as positive when the amount of immunoglobulin A was below 400mg/dL of ELISA value, 72 of 86 persons were proved as patients. Here, 83.7% of diagnostic sensitivity was shown. In case of diabetic patients without retinopathy, 22 of 45 persons were proved as patient. Here, 48.9% of diagnostic specificity was shown (see Table 2).

[Table 1] Average value of measuring immunoglobulin A in serum of healthy person and patient via ELISA

		Average IgA Conc. (mg/dL)
Healthy		217.6 ± 82.1
DM		457.5 ± 151.6
DMR	NPDR(non-proliferative)	244.4 ± 117.1
	PDR (proliferative)	278.6 ± 123.6

[Table 2] Judgement of diabetic retinopathy patient via ELISA standard 400mg/dL (cut off)

	Healthy	DM without retinopathy	DM with retinopathy
Over 400mg/dL	0	22	14
Below 400mg/dL	10	23	72
Total	10	45	86

[Industrial Applicability]

The present invention relates to a technology for easily diagnosing diabetic retinopathy which is a complication of diabetic mellitus. There has been no effective commercial diagnostic for diabetic retinopathy. Diabetic retinopathy has been diagnosed absolutely by oculists in hospital. It is impossible for diabetic patients to diagnose diabetic retinopathy in its early stage without regular ophthalmic examination and optical defect by subjective symptoms. The present diagnostic is characterized by simple blood test, and very effective in that the development of complications can be identified before ophthalmic examination. Particularly, the present invention is advantageous in its cheap cost and simple treatment for a

plurality of diabetic patients who take medical tests or consult physicians by adapting ELISA method using 96 wells which enable mass test. Also, the present invention is excellent in its accuracy and precision by using an immunochemical method. In conclusion, the present invention is effective for diagnosis of diabetic retinopathy in its early diagnosis and screening, and helpful for latent and early diabetic retinopathy patients in their decision of medication time, thereby delaying disease to severe diabetic retinopathy.

[What is Claimed is]

1. An Immunoglobulin A protein and an analogous protein or a protein fragment thereof described in SEQ ID NO:1 wherein the protein is effective for diagnosing diabetic retionpathy.

2. The protein fragment according to claim 1, wherein the protein fragment comprises a peptide sequence described in SEQ ID NO:2.

3. An antibody specifically binding the protein of claim 1 or 2.

4. A kit for diagnosing diabetic retinopathy comprising the antibody of claim 3.

5. The kit according to claim 4, further comprising enzyme peroxidase, alkaline phosphatase or biotin conjugated-anti-Immunoglobulin A antiboody.

6. A method for diagnosing diabetic retinopathy, comprising:

a) treating the antibody of claim 2 with a blood sample and an peroxidase, alkaline phosphatase or biotin conjugated-anti-Immunoglobulin A protein; and

b) measuring optical density of the compound,

wherein diabetic retinopathy is diagnosed when the measured value represents optical density (ELISA value) lower than normal one.

7. An Immunoglobulin A gene and an analogous gene of SEQ ID NO:3 for coding the protein of claim 1 or 2.

AMENDED CLAIMS
[received by the International Bureau on 09 September 2003 (09.09-03); original claims 1-7 replaced by amended claims 1-3 (1 page)]

[What is Claimed is]

1. (Amended) Immunoglobin A polypeptide for diagnosing Diabetic retinopathy wherein said polypeptide is selected from the group consisting of a polypeptide sequence SEQ ID NO: 1 and a peptide fragment of the polypeptide sequence SEQ ID NO:1.

2. (Amended) The protein of claim 1 wherein the peptide fragment comprises peptide sequence SEQ ID NO: 2.

3. (Deleted).

4. (Deleted).

5. (Deleted).

6. (Amended) A method for diagnosing diabetic retinopathy, comprising:

a) treating the antibody against polypeptide of claim 1 or 2 with a blood sample and an peroxidase, alkaline phosphatase or biotin conjugated-anti-Immunoglobulin A protein; and

b) measuring optical density of the compound,

wherein diabetic retinopathy is diagnosed when the measured value represents optical density (ELISA value) lower than normal one.

7. (Deleted).

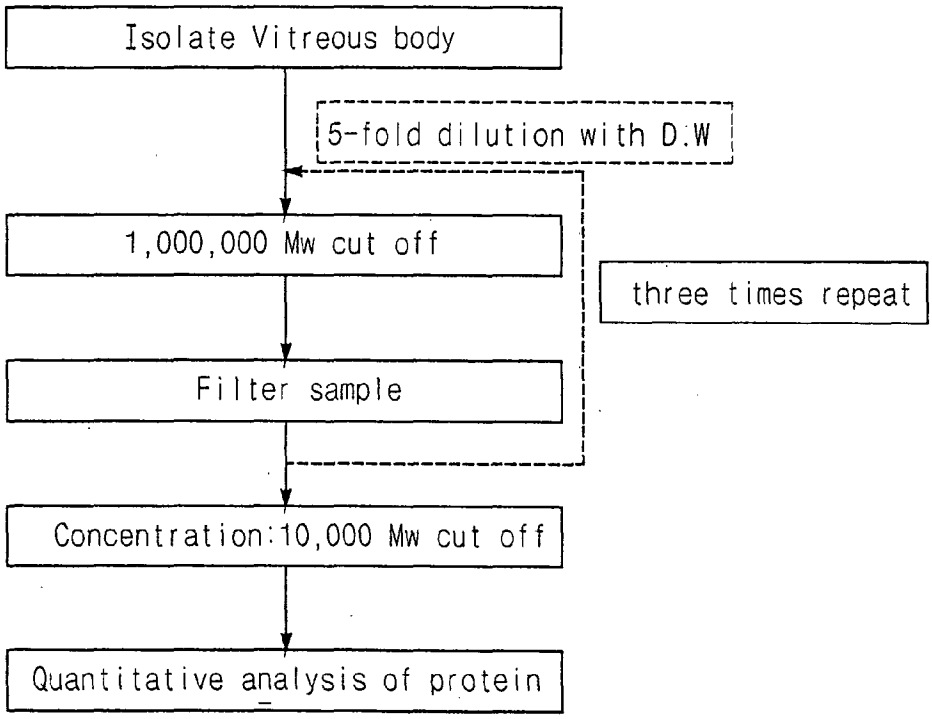

Isolate Vitreous body
5-fold dilution with D.W
1,000,000 Mw cut off
three times repeat
Filter sample
Concentration:10,000 Mw cut off
Quantitative analysis of protein

Fig.1

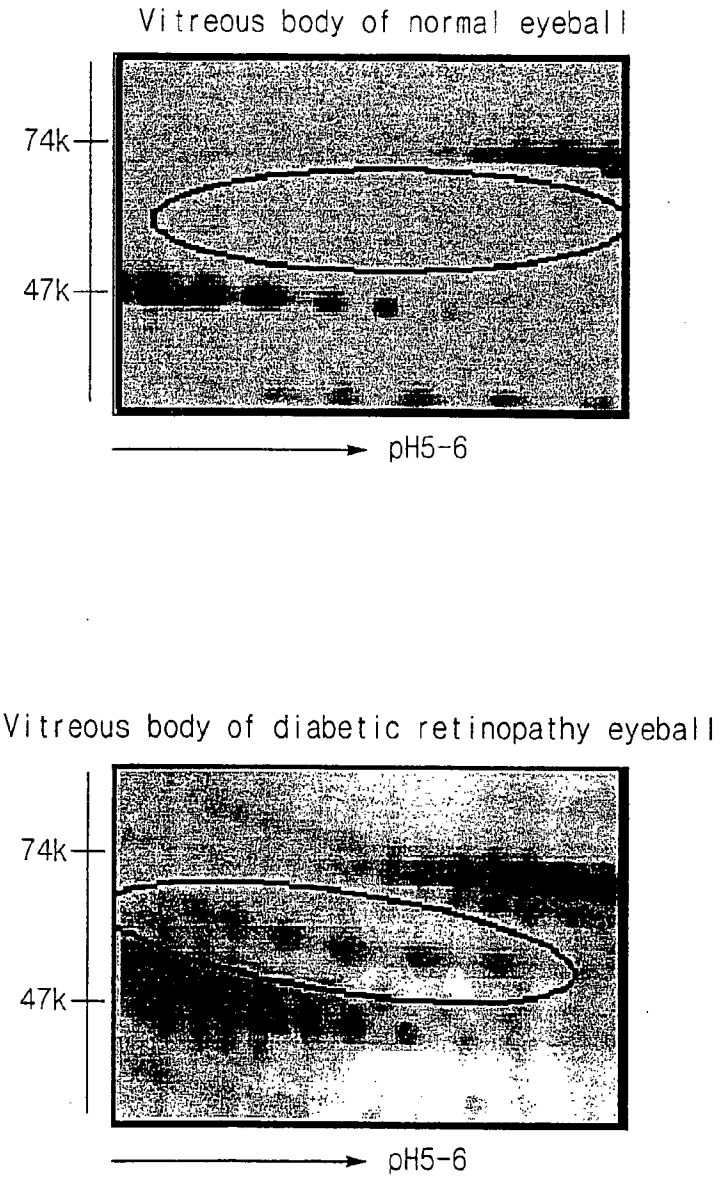

Vitreous body of normal eyeball
74k
47k
pH5-6
Vitreous body of diabetic retinopathy eyeball
74k
47k
pH5-6

Fig.2

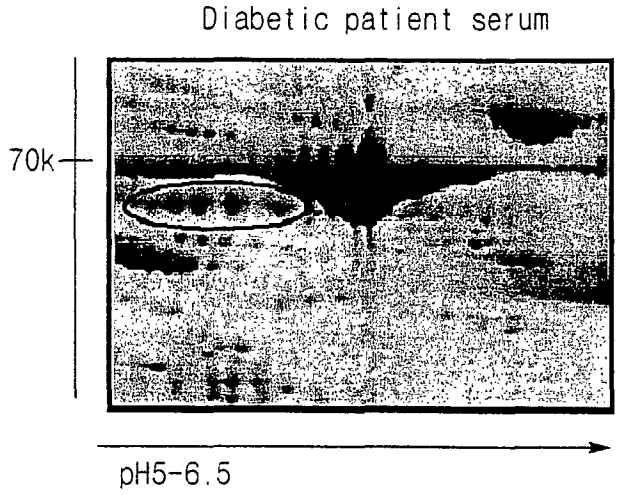
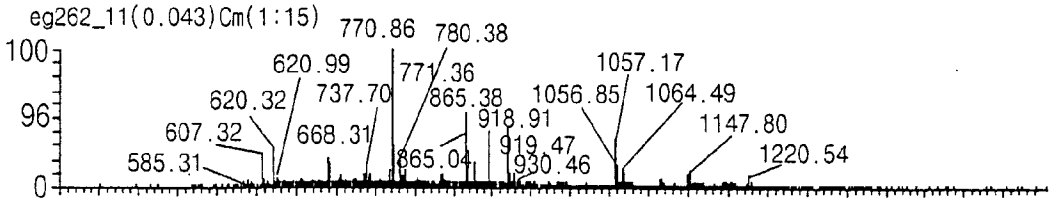

Diabetic patient serum
70k
pH5-6.5
Diabetic retinopathy patient serum
70k
pH5-6.5

Fig.3

A



B

Sample EG262

Observed	Mr(expt.)	Mr(calc.)	Delta	Miss	Peptide
607.32	1212.63	1212.63	0.01	0	WLQGSQELPR

Matching protein;
Ig A alpha 1 C region[Himo sapiens]

Ia alpa-2 chain C region [Homo sapiens]

Fig.4

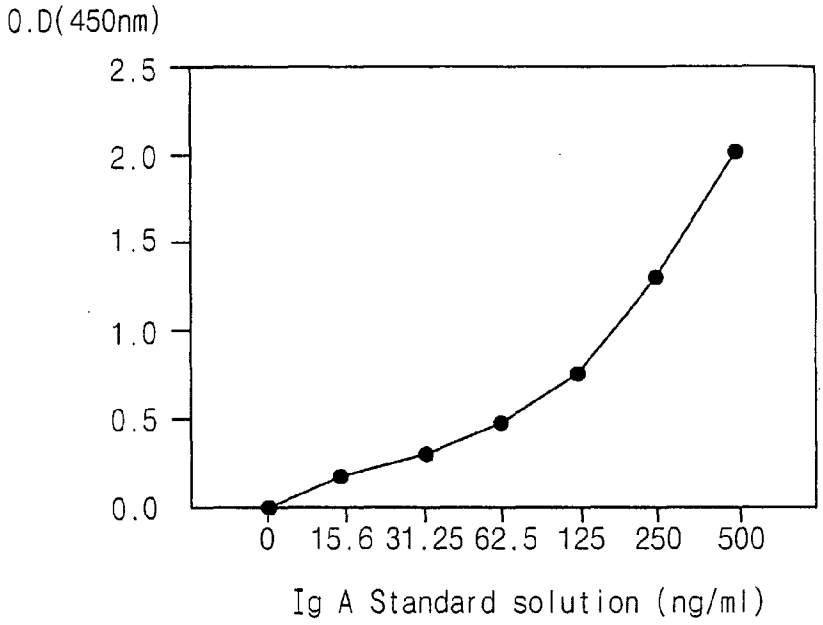

O.D(450nm)
2.5
2.0
1.5
1.0
0.5
0.0
0
15.6
31.25
62.5
125
250
500
Ig A Standard solution (ng/ml)

Fig.5

Sequence Listing

<110> EYEGENE INC.

<120> Protein for Diagnosing Diabetic Retinopathy

<150> KR102002041771
<151> 2002-07-16

<160> 4

<170> KopatentIn 1.71

<210> 1
<211> 353
<212> PRT
<213> Homo sapiens

<400> 1

Ala Ser Pro Thr Ser Pro Lys Val Phe Pro Leu Ser Leu Cys Ser Thr
1 5 10 15

Gln Pro Asp Gly Asn Val Val Ile Ala Cys Leu Val Gln Gly Phe Phe
20 25 30

Pro Gln Glu Pro Leu Ser Val Thr Trp Ser Glu Ser Gly Gln Gly Val
35 40 45

Thr Ala Arg Asn Phe Pro Pro Ser Gln Asp Ala Ser Gly Asp Leu Tyr
50 55 60

Thr Thr Ser Ser Gln Leu Thr Leu Pro Ala Thr Gln Cys Leu Ala Gly
65 70 75 80

Lys Ser Val Thr Cys His Val Lys His Tyr Thr Asn Pro Ser Gln Asp
85 90 95

Val Thr Val Pro Cys Pro Val Pro Ser Thr Pro Pro Thr Pro Ser Pro

Sequence Listing

100 105 110

Ser Thr Pro Pro Thr Pro Ser Pro Ser Cys Cys His Pro Arg Leu Ser
115 120 125

Leu His Arg Pro Ala Leu Glu Asp Leu Leu Leu Gly Ser Glu Ala Asn
130 135 140

Leu Thr Cys Thr Leu Thr Gly Leu Arg Asp Ala Ser Gly Val Thr Phe
145 150 155 160

Thr Trp Thr Pro Ser Ser Gly Lys Ser Ala Val Gln Gly Pro Pro Glu
165 170 175

Arg Asp Leu Cys Gly Cys Tyr Ser Val Ser Ser Val Leu Pro Gly Cys
180 185 190

Ala Glu Pro Trp Asn His Gly Lys Thr Phe Thr Cys Thr Ala Ala Tyr
195 200 205

Pro Glu Ser Lys Thr Pro Leu Thr Ala Thr Leu Ser Lys Ser Gly Asn
210 215 220

Thr Phe Arg Pro Glu Val His Leu Leu Pro Pro Pro Ser Glu Glu Leu
225 230 235 240

Ala Leu Asn Glu Leu Val Thr Leu Thr Cys Leu Ala Arg Gly Phe Ser
245 250 255

Pro Lys Asp Val Leu Val Arg Trp Leu Gln Gly Ser Gln Glu Leu Pro
260 265 270

Arg Glu Lys Tyr Leu Thr Trp Ala Ser Arg Gln Glu Pro Ser Gln Gly
275 280 285

Thr Thr Thr Phe Ala Val Thr Ser Ile Leu Arg Val Ala Ala Glu Asp

Sequence Listing

290 295 300

Trp Lys Lys Gly Asp Thr Phe Ser Cys Met Val Gly His Glu Ala Leu
305 310 315 320

Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp Arg Leu Ala Gly Lys Pro
325 330 335

Thr His Val Asn Val Ser Val Val Met Ala Glu Val Asp Gly Thr Cys
340 345 350

Tyr

<210> 2
<211> 10
<212> PRT
<213> Homo sapiens

<400> 2
Trp Leu Gln Gly Ser Gln Glu Leu Pro Arg
1 5 10

<210> 3
<211> 1059
<212> DNA
<213> Homo sapiens

<400> 3
gcaagcttga ccagccccaa ggtcttcccg ctgagcctct gcagcaccca gccagatggg 60

aacgtggtca tcgcctgcct ggtccagggc ttcttccccc aggagccact cagtgtgacc 120

tggagcgaaa gcggacaggg cgtgaccgcc agaaacttcc cacccagcca ggatgcctcc 180

Sequence Listing

ggggacctgt acaccacgag cagccagctg accctgccgg ccacacagtg cctagccggc 240

aagtccgtga catgccacgt gaagcactac acgaatccca gccaggatgt gactgtgccc 300

tgcccagttc cctcaactcc acctacccca tctccctcaa ctccacctac cccatctccc 360

tcatgctgcc acccccgact gtcactgcac cgaccggccc tcgaggaccc gctcttaggt 420

tcagaagcga acctcacgtg cacactgacc ggcctgagag atgcctcagg tgtcaccttc 480

acctggacgc cctcaagtgg gaagagcgct gttcaaggac cacctgaccg tgacctctgt 540

ggctgctaca gcgtgtccag tgtcctgtcg ggctgtgccg agccatggaa ccatgggaag 600

accttcactt gcactgctgc ctaccccgag tccaagaccc cgctaaccgc caccctctca 660

aaatccggaa acacattccg gcccgaggtc cacctgctgc cgccgccgtc ggaggagctg 720

gccctgaacg agctggtgac gctgacgtgc ctggcacgtg gcttcagccc caaggatgtg 780

ctggttcgct ggctgcaggg gtcacaggag ctgccccgcg agaagtacct gacttgggca 840

tcccggcagg agcccagcca gggcaccacc accttcgctg tgaccagcat actgcgcgtg 900

gcagccgagg actggaagaa gggggacacc ttctcctgca tggtgggcca cgaggccctg 960

ccgctggcct tcacacagaa gaccatcgac cgcttggcgg gtaaacccac ccatgtcaat 1020

gtgtctgttg tcatggcgga ggtggacggc acctgctac 1059

<210> 4
<211> 30
<212> DNA
<213> Homo sapiens

Sequence Listing

<400> 4

tggctgcagg ggtcacagga gctgccccgc 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00544

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C07K 16/18, C07K 16/06, C07K 14/47, C12N 15/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

NCBI PubMed database, Esp@cenet database "Immunoglobulin A and diabetic retinopathy"

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Genbank Accession No. P01876 'Ig alpha-1 chain C region' 1 February 1991 (01.02.1991)	1, 2
X	Genbank Accession No. AJ294729 'Homo sapiens partial mRNA for immunoglobulin heavy chain constant region alpha 1 (IGHA1 gene)' 9 February 2001 (09.02.2001)	7
Y A	LEE E.Y. et al. 'Immunoglobulin A nephropathy in patients with non-insulin dependent diabetes mellitus' In: J Korean Med Sci, 1999, Vol.14, pp582-585 See the whole document	3-5 1, 2, 7
Y A	PEEBLES R.S. Jr. et al. 'IgA, IgG and IgM quantification in bronchoalveolar lavage fluids from allergic rhinitics, allergic asthmatics, and nomal subjects by monoclonal antibody-based immunoenzymetric assays' In: J Immunol Methods, 1995, Vol.179, pp77-86 See the whole document	3-5 1, 2, 7
A	STOLWIJK T.R. et al. 'Analysis of tear fluid proteins in insulin-dependent diabetes mellitus' In: Acta Ophthalmol, 1994, Vol.72(3), pp357-362 See the abstract	1-5, 7

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 JUNE 2003 (28.06.2003)	30 JUNE 2003 (30.06.2003)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

KWON, Oh Hee

Telephone No. 82-42-481-5597

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR03/00544

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 6
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 6 is directed to the diagnostic method practiced on the human or animal body and is a subject matter which the International Search Authority is not required to search under Article 17(2)(a)(i) and Rule 39.1(iv) PCT.

2. [ ] Claims Nos.:
because they relate to part of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Search Authority found multiple inventions in this international application, as follows:

1. [ ] As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be established without effort justifying an additional fee, this Authority did not invite payment of any addition fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.